



PATENT

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Dated: June 10, 2008

BY: Rodney DeKruif
Rodney D. DeKruif

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Emrick et al.)
Serial No: 10/643,015)
Attorney Docket No. 7163
Filed: August 18, 2003)
For: PYRIDINE AND)
RELATED LIGAND)
COMPOUNDS,)
FUNCTIONALIZED)
NANOPARTICULATE)
COMPOSITES AND)
METHODS OF)
PREPARATION)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RULE 131 DECLARATION OF HABIB SKAFF

1. I, Habib Skaff, am a co-inventor with regard to the invention (the "Invention") disclosed and claimed in the above-entitled application (the "Application"). I make this declaration in support of the Application and, in particular, to antedate a reference cited against the Application.

2. The Invention claimed in the Application was completed before the effective date of application serial number 10/219,440 (*i.e.*, the Dubertret

reference). More specifically, the Invention was conceived and with due diligence reduced to practice, in this country--the United States of America, prior to the effective date of the Dubertret reference.

3. This Declaration, and prior invention, is supported by copies of pertinent pages from my laboratory research notebook, entries to which I contemporaneously signed and dated and were witnessed by co-inventor, Todd S. Emrick. Date redacted copies of the aforementioned notebook pages are provided collectively as Exhibit A and incorporated herein by reference. These documents establish that the Invention was made at least as early as June 1, 2002, which is a date earlier than the effective date of the Dubertret reference.

4. More specifically, as part of my graduate research work with co-inventor Emrick, I prepared composites of a metallic nanoparticulate component coupled to a polymeric ligand component. For purposes relating to our research, we referred to such a nanoparticulate as a nanocrystal, abbreviated "Nc". Preparation of such a nanoparticulate, Nc, composite is evidenced on page 37 of Exhibit A, and the composite recorded therein was prepared at least as early as June 1, 2002.

(a) Representing a range of available nitrogen-containing moieties, I used a pyridinyl group to couple the ligand and nanoparticulate components. Coupling of such ligand and nanoparticulate components is evidenced on page 37 of Exhibit A, and the coupling recorded therein was achieved at least as early as June 1, 2002.

(b) Representing a range of available nanoparticulate components, I used CdSe. Use of such a nanoparticulate is evidenced on pages 37-38 of Exhibit A, and the nanoparticulate recorded therein was used at least as early as June 1, 2002.

(c) Representing a range of available polymers, I used poly(ethylene glycol) to prepare such a polymeric ligand component. Preparation of such a ligand component, including coupling to a pyridinyl moiety, is evidenced on pages 14-15, 23, 25 and 37 of Exhibit A, and the ligand recorded therein was prepared at least as early as June 1, 2002.

(d) Representing a range of available terminal functional groups, I chose a hydroxy group to terminate poly(ethylene glycol). Preparation of such a hydroxy-terminated polymeric ligand component, and subsequent coupling with a nanoparticulate, is evidenced on pages 15 and 37 of Exhibit A, and the ligand recorded therein was prepared and used at least as early as June 1, 2002.

5. As a related part of my graduate research with co-inventor Emrick, I also prepared systems for nanoparticulate dispersion. As part of such a system, I prepared a composite of a metallic nanoparticulate component (Nc) and a ligand component in a liquid medium: a representative nanoparticulate component, CdSe, was coupled to a tri-n-octyl phosphine oxide (TOPO) ligand component, then dissolved in tetrahydrofuran (THF). Preparation of such a composite of a ligand component in a liquid medium is evidenced on page 38 of Exhibit A, and

the composite in liquid medium recorded therein was prepared at least as early as June 1, 2002.

(a) The system I prepared also included another ligand component in another liquid medium. Representative of many available ligands and as described above, I used a ligand of poly(ethylene glycol) with a pyridinyl coupling moiety, and I showed this ligand to be soluble in water. Ligand preparation and water solubility is evidenced on page 15 of Exhibit A, and the ligand recorded therein was prepared and dissolved at least as early as June 1, 2002.

(b) To demonstrate this dispersion system, I precipitated the CdSe-TOPO composites out of THF solution. To demonstrate greater nanoparticulate affinity for another ligand component, the CdSe-TOPO composites were redissolved in a water medium containing pyridinyl/poly(ethylene glycol) ligand components. With dissolution of the nanoparticulates in water, I showed that the pyridinyl/poly(ethylene glycol) ligand components have greater affinity for the CdSe nanoparticulates than the TOPO ligand components. This dispersion system, showing greater nanoparticulate affinity of one ligand component over another, is evidenced on page 38 of Exhibit A, and the system for nanoparticulate dispersion recorded therein was demonstrated at least as early as June 1, 2002.

I hereby declare that: All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be

true; that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code; and that willful false statements may jeopardize the validity of the Application or any patent issuing thereon.

Date 6/5/08

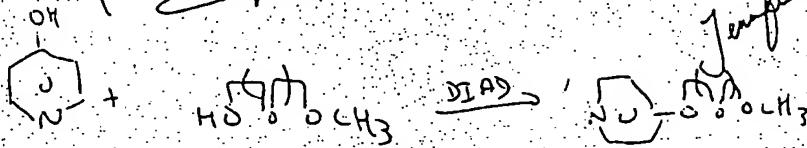


Habib Skaff

Methylated

Taylor

Kurt E. Bl
Jeffrey J. Sauer 3



Reagents

95 ① 2g, 0.022 mol

②

250 ③ m-Py FDO 14.25 g, 0.019 mol

262 ④ Ph₃P 6.25 g, 0.024 mol

212 ⑤ DIAD 4.84 g, 0.024 mol (4.72 mL)

⑥ THF (dil) 300mL 250mL

Procedure

① Ph₃P + THF loaded into 2-neck flask & stirred under N₂ 0.1 L.

② DIAD added via syring & stirred for 1/2 hr.

③ phenol & alcohol added & stirred

④ reacted over night

⑤ distilled off THF

⑥ added of DIAD & ether & washed w/ Et₂O

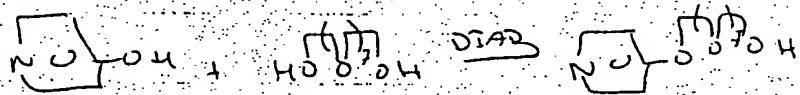
⑦ extracted product out w/ CH₂Cl₂ out

of Ag phase $\xrightarrow{\text{PbSO}_4}$, reduced

$\xrightarrow{\text{NMR}}$ shows some CH_2 $\xrightarrow{\text{triflavin redissolving in dil. dioxane}}$ work

(5M NaOH) basic solution is precipitating into CH₂Cl₂ (cold)

$\xrightarrow{\text{column eluting w/ Et₂O}}$ in meth (7:3:0), (7:2:1)

Reagents

45 ① $\text{Ph}_3\text{P} + \text{Zn}, 0.01\text{ mol}$

400 ② $\text{HOPO}_4, 22\text{ g}, 0.055\text{ mol}$
 $p = 1.03$

202 ③ DIAE 2.63g, 2.55ml 0.013mol

262 ④ Ph_3P 3.41g, 0.03
 $\text{THF}(\text{dry})$
~~3 250 mL~~

Procedure

① Ph_3P + THF loaded into 3-neck 500mL round-bottom flask
 stirred @ rt under N_2

② DIAE added via syring & stirred for 1hr.

③ Phenol + Et₂O diethyl added & stirred

→ reacted over night

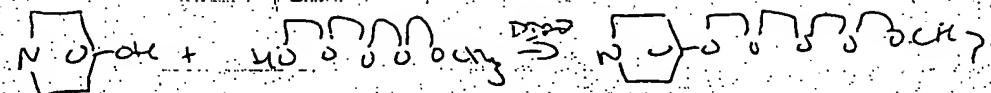
- volatilized off all THF $\xrightarrow{\text{rotovap}}$

extracted w/ H_2O → then aqueous wash

w/ CH_2Cl_2 → too difficult to purify by column

→ rotovap'd off CH_2Cl_2 → dissolved in H_2O ,
 washed w/ cold, then Toluene → doesn't work well!

try ~~add NaOH~~ acidifying aqueous to make pyridine salt
 which will not be soluble in



Reagents

(1) $\text{N}_2\text{-OAc}$ 5g, 0.055 mol

(2) m-Ts 5.632g, 0.044 mol

(3) Ph₃P 13.1g, 0.05 mol

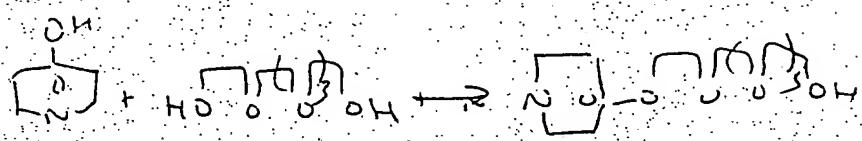
(4) DDA 10.1g, 0.05 mol, 9.85 mL

(5) THF (dry) ~~400 mL~~ 400 mL

Procedure

~~DDA~~ Ph₃P & THF loaded into 2-neck flask
& stirred under N₂-Q.C.

- ② DDA added via syring & stirred for 1 hr.
- ③ q.lentol & alcohol added & stirred overnight



Reagents

95



4g, 0.042 mol

300 ② Hg

31.58g, 0.105 mol

262 ③ Ph₃P

13.1g 0.045

(11.1 20) ④ DSA

10.1g, 0.05 mol, + 9.85 mL

⑤ THF

500 mL

Procedure

① phenol, Ph₃P, DSA, i-THF loaded in 2-neck
& stirred @ r.t under N₂ for ½ hr.

② diol added → stirred overnight

→ decanted at THF

→ brine/CH₂Cl₂ ② CH₂Cl₂: H₂O (90:20)
→ vcn column eluted w/ ④ CH₂Cl₂: H₂O (7:2:1)

→ redistilled off unreacted diol @ 224°C @

④ 600 mbar → didn't work well

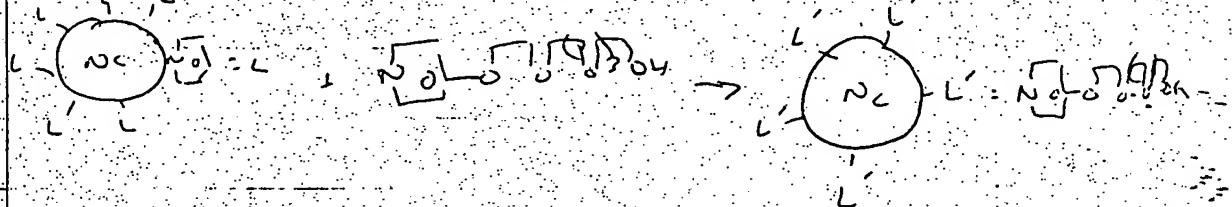
→ vcn column in CH₂Cl₂: H₂O (78:20:2),
(75:20:5), (80:20:10)

Mallinckrodt

John
Elliott

Jennifer L. Brown
Trite Biologics

Exchange to bis-monophenyl ether



Reagent

- ① Dry ice Nc ~40mg
- ② $\text{Nc} \text{---} \text{O} \text{---} \text{C}_6\text{H}_4$ 600mg
- ③ THF (dry) 3mL
- ④ DIW 6mL

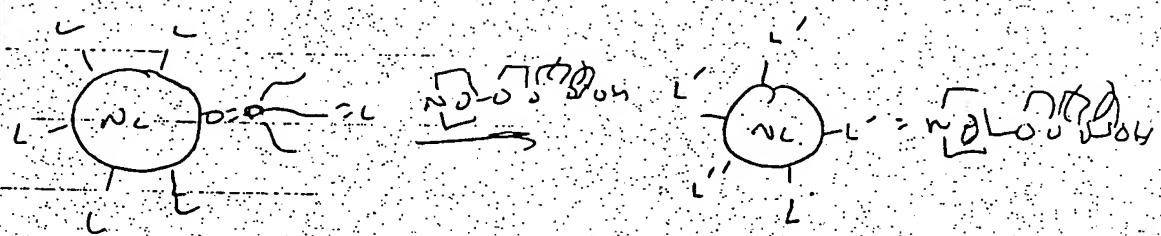
Procedure

A) ① 20mg Nc dispersed in solution at 30deg
new ligand in THF \rightarrow predictably went
into solution

② dried under N₂ flow and added 3mL
DIW \rightarrow ^{not} _{some} went into solution \rightarrow centrifuged
③ 20mg Nc dispersed in solution at 30deg
new ligand in 3mL DIW \rightarrow Nc went into
solution \rightarrow centrifuged Jennifer L. Sonne

Jeff Shaffer

Jennifer L. Sonne K.E. Bi



Reagents

- ① TOPD covered NC ~ 15mg
- ② N_2-O (gas) 320 mg
- ③ THF (dry) 3 mL

Procedure

- ① Pre-cooled as ^{usual} & dashed w/
MeOK 3 times
- ② dried over N_2 flow
- ③ redissolved in new ligand in THF and
allowed to stand over head of N_2 overnight
- ④ distilled at $\frac{1}{2}$ THF \rightarrow precipitated w/
hexane \rightarrow all NC precipitated
- ⑤ washed w/ hexanes \rightarrow centrifuged \rightarrow
redissolved in TOPH_2O

Bell & Howell K. E. Bill

T. E. Bill

Janice L. Sorenson